



THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Malmros <i>et al.</i>)	
)	
Serial No.: 09/306,662)	Group Art Unit: 1642
)	
Filed: May 5, 1999)	Examiner: S. Rawlings
)	
Attorney Docket No: 111499.121)	

For: METHOD OF IN SITU DIAGNOSIS BY SPECTROSCOPIC ANALYSIS OF
BIOLOGICAL STAIN COMPOSITIONS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MICHAEL R. HAMBLIN PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Michael R. Hamblin, Ph.D., hereby declare that:

1. I received my Ph.D. in Synthetic Organic Chemistry from Trent Polytechnic, U.K. and have spent more than 27 years involved in the fields of enzyme chemistry, synthetic organic chemistry, and photodynamic therapy. I was a post-doctoral researcher at the New University of Ulster, U.K. from 1976 to 1978, the University of Edinburgh, U.K. from 1978 to 1979, and the Heriot-Watt University, U.K. from 1979 to 1982. I then worked as a Research Fellow/Associate at the University of Cambridge, U.K. from 1982 to 1984, Leicester University, U.K. from 1984 to 1987, and the University of Dundee, U.K. from 1990 to 1994, in the Departments of Biochemistry and Chemistry. I was an Instructor at the Wellman Laboratories of Photomedicine at Harvard Medical School from 1994 to 1997 and since then I have been an Assistant Professor of Dermatology at Harvard Medical School. I have authored or co-authored more than 55

journal articles, reviews, and book chapters. I have also authored 65 Abstracts. Of these publications, about 30 are related to dyes and biological staining. I am also a named inventor on 4 issued U.S. patents as well as 2 pending patents. My curriculum vita along with a list of publications, presentations, and patents is enclosed hereto as Attachment A.

2. I have acted as a consultant for AzurTec, Inc. ("AzurTec") in connection with its submissions to the FDA; therefore, I am very familiar with AzurTec's technology.

3. I have reviewed and analyzed U.S. Patent Application No. 09/306,662; the Final Office Action dated June 8, 2004, the Office Action dated December 18, 2002, the Office Action dated November 26, 2001, U.S. Patent No. 5,784,162 to Cabib et al., the Vaezy et al. reference (*Journal of Microscopy*, 163: 85-94, 1991), the Marchesini et al. reference (*Photochemistry and Photobiology*, 55: 515-522, 1992), and the Tuite et al. reference (*Journal of Photochem. and Photobiol. B: Biol.*, 21: 103-124, 1993), which were cited by the Examiner in the Final Office Action.

4. In the Office Action of June 8, 2004, the Examiner has rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, first paragraph, as he asserts that the specification does not describe the subject matter of these claims in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner further opines that the terms "the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissues or cells" and "the degree of the metachromatic shift of the dye from a library" lack sufficient antecedent basis in the specification. In my opinion, one of skill in the art would understand that these terms are

supported by the specification and would also understand that the inventors had possession of the claimed invention. The basis for my opinion is set out in paragraphs 5-8 below.

5. Page 8, line 29 to page 9, line 7 (paragraph [0030] of the published application), of the specification states:

The (reflectance) spectroscopic analysis of lesions that stain with toluidine blue or with other biological stains or dyes, or with a combination of such stains or dyes...allow for a differential diagnosis of the underlying disease, or disease state of the stained lesion. Cells displaying various stages of metaplasia stain differentially...which is then correlated to the spectrum with a high degree of specificity. This is accomplished by comparing the reflectance spectrum of the stained tissue or lesion with a 'library' or composite of spectrums from lesions that have been similarly stained and subsequently diagnosed by conventional or classical histochemical methods.

6. Based on the language recited above, as well as the additional disclosure of the specification, specifically page 8, lines 9-16 (paragraphs [0027-0028] of the published application) and page 22, lines 1-5 (paragraph [0070] of the published application), it is my view that a person of ordinary skill in the art, for example, a Mohs surgeon or a pathologist, would understand that the inventors had possession of an invention directed to a method of diagnosing the degree of metaplasia in biological tissue or cells by obtaining a reflected light spectrum of the stained tissue or cells and comparing the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue.

7. It is, therefore, my opinion that the patent specification provides support for the claim which recites, "A method for diagnosing dysplasia, pre-cancer or cancer in situ in biological tissue or cells of a living organism, comprising [...] comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with

the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells," as would be understood by a person of ordinary skill in the art.

8. Thus, it is my opinion that one skilled in the art, given the description in the specification, in conjunction with the knowledge and skill in the art at the time of filing, would understand that the inventors had possession of the claimed invention.

9. The Examiner has also rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, second paragraph, as he asserts that these claims are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner also opines that the phrase, "comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library" is vague. The Examiner believes that it is not clear what aspect or characteristic of the spectrum is to be compared with the library of the previously obtained spectra as he asserts that the terms "the metachromatic shift" and "the degree of the metachromatic shift" appear not to be defined in the specification.

10. I am familiar with the phenomenon of metachromasia where, for example, a dye stains certain cell components a different color than the original color of the dye. It is also known to those of skill in the art that this metachromasia is quantitated by measuring the extent of the metachromatic shift by comparing the intensity of the light in a desired light spectrum between, for example, two or more specific wavelengths.

11. Since one of ordinary skill in the art would know how to determine the degree of the metachromatic shift (as described above), one of ordinary skill in the art would also know

how to compare these degrees in metachromatic shifts. The metachromatic shift is a change in wavelengths measured in nanometers while the intensity is typically measured in absorbance units. Therefore, the sample tissue or cells would be illuminated with light of a known spectrum (i.e., known intensity at every wavelength), and the spectrum of the remitted light would be measured to determine how much light was absorbed at every wavelength. The measured wavelength and intensity of the stained tissue or cells could then be compared to measurements from a library of previously obtained spectra of similarly stained tissue or cells that were illuminated by the same piece of equipment.

12. The Examiner also asserts that the phrase "with a library of previously obtained spectra of similarly stained tissue or cells" is vague and indefinite because it cannot be determined from which similarly stained tissue or cells said library of previously obtained spectra is to be obtained prior to steps (a)-(d), and from what source said similarly stained tissue and cells are to be derived.

13. Upon reviewing the specification, it is my opinion that one of ordinary skill in the art would understand that the library of previously obtained spectra would include those cells and/or tissues that are the subject of the particular analysis and would be obtained from individuals that may exhibit the particular cellular abnormality. For example, if a diagnosis is to be made of skin cancer, it would be my understanding that analyses/spectra of skin cancer cells/tissues obtained from individuals with skin cancer could serve as the particular reference. For instance, one of skill in the art would understand that the library could be composed of skin cancer cells/tissues obtained from a wide variety of skin cancer types including, for example,

blue rubber bleb nevus, leiomyoma, eccrine poroma/eccrine spiradenoma, neuroma, dermatofibroma/dercum's disease (adiposis dolorosa), angioliipoma, neurilemoma, endometrioma, glomus tumor, granular cell tumor, acrochordon (skin tag, fibroepithelial polyp), actinic cheilitis, actinic keratosis, aggressive digital papillary adenocarcinoma, basal cell carcinoma, Birt-Hogg Dube syndrome, Bowen's Disease, chondroid syringoma (mixed tumors), chronic papillomatous dermatitis, Cowden's Syndrome, cylindroma of the skin (turban tumors), cysts of the skin, eccrine carcinoma (porocarcinoma, adenoid cystic carcinoma), epidermodysplasia verruciformis, extramammary Paget disease, Favre-Racouchot disease (nodular elastosis with cysts and comedones), keratoacanthoma, keratoses (seborrheic keratosis, acanthomas), merkel cell carcinoma, metastatic tumors to the skin, microcystic adnexal carcinoma, mixed tumors (chondroid syringoma), myelomeningocele, nevoid basal cell carcinoma syndrome (Gorlin Syndrome), nevus sebaceus, onychomatricoma, pilomatricoma, poroma (eccrine poroma), Sebaceous Carcinoma (sebaceoma, sebaceous adenoma, sebaceous epithelioma), soft tissue tumors (neurofibroma, granular cell tumor), spiradenoma (eccrine spiradenoma), squamous cell carcinoma, syringocystadenoma papilliferum, syringoma, trichoblastoma, trichoepithelioma, tricholemmal tumors, tumor of the follicular infundibulum, verruca vulgaris (warts), and basal cell carcinoma (including morpheaform, sclerosing, infiltrative, and nodular). Analyses/spectra of healthy skin cells/tissues could also form part of the library for this particular analysis.

14. It is also my opinion that one of ordinary skill in the art would understand that this concept of a "library of previously obtained spectra of similarly stained tissue or cells" is further

supported by the specification at page 8, line 29 to page 9, line 7 (paragraph [0030] of the published application), which states:

The (reflectance) spectroscopic analysis of lesions that stain with toluidine blue or with other biological stains or dyes, or with a combination of such stains or dyes...allow for a differential diagnosis of the underlying disease, or disease state of the stained lesion. Cells displaying various stages of metaplasia stain differentially...which is then correlated to the spectrum with a high degree of specificity. This is accomplished by comparing the reflectance spectrum of the stained tissue or lesion with a 'library' or composite of spectrums from lesions that have been similarly stained and subsequently diagnosed by conventional or classical histochemical methods.

15. The Examiner also asserts that claims 1, 5, and 7-11 are vague and indefinite because of claim 1's recital of the phrase "correlating the reflected light spectrum with a disease state." The Examiner opines that it cannot be ascertained how correlating the reflected light spectrum with a disease state leads to a diagnosis of dysplasia, pre-cancer, or cancer in a living organism, since it cannot be determined how the spectrum of the reflected light and disease state are related.

16. It is my opinion that one of ordinary skill in the art would understand that metachromasia occurs because of the dye's interaction with each individual cell component (i.e., nucleic acids, lipids, proteins, and polysaccharides), resulting in the differential staining of these individual cell components. This concept is supported throughout the specification, for example, at page 3, line 20 to page 4, line 25 (paragraphs [0006-00010] of the published application), which describes various cell components that are stained a different color than the original color of the dye. Furthermore, at page 4, line 27 to page 5, line 2 (paragraph [0011] of the published application), the specification describes the potential mechanisms underlying this metachromasia in stating:

There are three mechanisms (not mutually exclusive) of staining that have been suggested by the research to date: 1) mucopolysaccharide staining with metachromasia (a concomitant shift in the absorption spectra of the phenothiazine compound), 2) enhanced nuclei and nucleoli staining (RNA and DNA rich) associated with enhanced proliferation of these organelles in pre-cancerous and cancerous cells and, 3) enhanced staining of the mitochondria of metaplastic (dysplastic, pre-cancerous, and cancerous) cells.

17. In addition, the specification at page 8, lines 14-20 (paragraph [0028] of the published application) further describes how dysplastic, pre-cancerous, and cancerous lesions are differentially stained by the metachromatic dyes in stating:

The histochemical pathology of dysplastic, pre-cancerous, and cancerous lesions that would expected to be stained with any of the thiazine dyes, will vary as to the degree of metachromasia within the cell tissue layer. This depends on the variation in mucin production, aberration of nuclei, nucleoli, and mitochondrial organelle distribution, as well as changes in cell membrane permeability, charge structure and membrane transport properties, etc. with the various cells types associated with each diagnosis and the stage of metaplasia or cell transformation.

18. Furthermore, the specification at, for example, page 8, line 29 to page 9, line 7 (paragraph [0030] of the published application), and page 22, lines 1-5 (paragraph [0070] of the published application), clearly explains how one can compare the extent of the metachromatic shift in the test sample spectra with that obtained from the library of previously obtained spectra of similarly stained tissues or cells. The specification also describes how the samples upon which the library is based have been previously diagnosed by conventional techniques. Therefore, a correlation as to the disease state of the test sample may be made by such comparison of the metachromatic shift of the test sample with that of the reference sample.

19. The Examiner has also rejected claims 1, 5, and 7-11 as being indefinite because claim 1 recites the term "similarly stained tissue or cells". The Examiner opines that it cannot be ascertained how similarly the different tissue or cells may be stained.

20. In order to compare staining results from separately stained tissue/cell samples, it is common knowledge to one of ordinary skill in the art that the same standardized protocol would have to be used to stain the two samples. For instance, the same dye and solvents would have to be used at substantially the same concentrations and staining should occur for substantially the same amount of time. Since it is always scientifically possible for a protocol to be standardized, any difference in the staining would be due to differences between the particular cell or tissue samples that are being stained rather than any difference in the protocols.

21. It is my opinion that one skilled in the art, given the guidance provided in the specification, in conjunction with the knowledge and skill in the art at the time of filing, would understand the extent of the subject matter that Applicant regards as the invention because it is distinctly claimed.

22. In the Office Action of June 8, 2004, claims 1, 5, 7-11, and 20 have also been rejected under 35 U.S.C. § 102 as being anticipated by Cabib et al. (U.S. Patent No. 5,784,162), as evidenced by Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al. (*Photochemistry and Photobiology*, 55: 515-522, 1992). The Examiner has relied on Cabib's description of spectral imaging methods for *in situ* medical diagnosis and treatment comprising preparing a sample to be imaged, viewing a sample through an optical device optically connected to a spectrometer, collecting and measuring incident light using a detector and collecting and

interpreting data using a mathematical algorithm, in a prior art rejection. In the Office Action of November 26, 2001, the Examiner also opines that 1) “numerous examples of *in situ* analyses of cells and/or tissues to either classify and/or diagnose cellular abnormalities in said cells and/or tissues are provided” in Cabib et al., and Examples 1, 6, 7 and 8 are pointed to with particularity; 2) Cabib et al. “discloses that a metachromatic dye, such as Azure-B, which is a thiazine dye, can be used to practice the prior art methods (see Example 2, column 43, line 10);” 3) “the sample of tissue or cells to be analyzed [in Cabib et al.] is prepared by staining with either Romanowsky-Giemsa stain, haematoxylin-eosin stain, or May-Grunwald-Giemsa stain (see claim 59), each of which are compositions comprising thiazine dyes;” 4) Cabib et al. teaches “that a spectral component may ‘correlate well with what is called the purple Romanowsky-Giemsa complex,’” and 5) “an objective of the prior art invention is to distinguish cancer from healthy or otherwise diseased tissue or cells (column 6, lines 27-33).”

23. The present invention relates to methods for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissue or cells of a living organism that include utilization of spectroscopic methods to analyze the metachromatic properties of various dyes in abnormal (e.g., dysplastic, pre-cancerous and cancerous) and normal cells.

24. The inventors of the present invention have surprisingly discovered that the extent of the metachromatic shift observed in a dye from stained tissue or cells can be used to differentiate, for example, the aforementioned abnormal cells and/or tissues from normal cells and/or tissues.

25. The metachromatic shift of a dye observed in the reflected light spectrum from a dye-stained test sample may be compared to the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells wherein the diagnosis of the disease state of the reference cells or tissue upon which the previously obtained spectra are based was confirmed by conventional histopathology methods, such as histochemical methods. Thus, quicker, more precise diagnoses may be made according to the present method compared to existing methods in the art.

26. In my opinion, one of ordinary skill in the art, at the time U.S. Patent Application No. 09/306,662 was filed, would understand that Cabib et al. does not describe or suggest quantifying the extent of metachromatic shift of a metachromatic dye in a stained test sample and making a correlation with a dysplastic, pre-cancer or cancer disease state using this information as recited in the pending claims. Cabib et al. is an extremely broad and general disclosure which describes numerous spectral imaging methods for biological research and medical diagnostics. However, nowhere in Cabib et al. is there any mention or suggestion of metachromasia or the utilization of the metachromatic shift using the methods of the present invention.

27. For instance, the Cabib et al. reference primarily relates to detecting spatial organization and quantifying cellular and tissue natural constituents and does not teach or suggest methods of diagnosing dysplasia, pre-cancer or cancer by quantifying, for example, the metachromatic shift of a metachromatic dye. Cabib et al. are relying on the inherent spectra of biological components in making a determination of cancer, not use of dyes as described in applicants' specification. For instance, at column 1, lines 17-21, Cabib et al. states that [t]he

methods of the present invention can be used to detect spatial organization (i.e., distribution) and to quantify cellular and tissue natural constituents, structures, organelles and administered components such as tagging probes (e.g., fluorescent probes) and drugs....” It is further stated in column 6, lines 36-39, of Cabib et al. that “[t]he method further enables the identification and spatial mapping of proteins, sacharides [sic], AND+ [sic] and NADH, collagen, elastin and flavin, and various additional metabolic mediators within cells and/or tissues.” Additionally, it is stated in column 6, lines 27-33, that “[a]nother objective of the present invention is to map in a quantitative way white light, ultraviolet or laser-induced emission spectra from biological components (e.g., oxygenated and deoxygenated hemoglobin in retinal blood vessels and or melanin pigmentation level in the retina) and, to distinguish cancer from healthy, or otherwise diseased tissue or cells.” Based on these statements, as well as the rest of the disclosure in the Cabib et al. reference, it is my opinion that Cabib et al. are relying on the spectra of the biological components in making a determination of cancer.

28. Furthermore, in the Office Action of December 18, 2002, the Examiner has also asserted that Cabib et al. (a) teaches “morphometric spectral image analysis enables evaluation of subtle cytological and histological features to yield useful ultrastructural and medical information for diagnostic and prognostic evaluation,” (b) discloses “[s]ince various malignancies are also characterized by unique developmental features, the SpectraCube™ system and the methods of the present invention can be adopted to monitor these characterizing features and thus to assist in for example early diagnosis (e.g., existence and stage) of such malignancies;” and (c) discloses an example (Example 8) in which the SpectraCube™ system

and the methods of the claimed invention were used to differentiate a cancerous cell from a normal cell.

29. As with the entirety of the Cabib et al. disclosure, the description of the SpectraCube™ system is too broad and general as to provide any suggestion or description of the methods of the present invention. Therefore, one of ordinary skill in the art would understand that The SpectraCube™ system of Cabib et al. does not describe or suggest the utilization of metachromasia in making a diagnosis of dysplasia, pre-cancer or cancer in situ for the reasons discussed below.

30. Cabib et al. describes the use of dyes as contrast agents to visualize structures (e.g., to look at biological components and/or their spectrum) and does not describe or suggest the use of metachromasia for any diagnostic purpose. For instance the "morphometric spectral image analysis" relates to applying spectral imaging to improve the quantitative measurement of the size, shape and textural features of cells as demonstrated in Example 2. In Example 2, the dye is used as a contrast agent to visualize cellular structures. When a dye is used as a contrast agent, the intensity of the light absorbed by the stained sample is measured in order to differentiate the various cellular components that are stained at varying intensities by the same dye. On the other hand, the analysis of the metachromatic shift of a dye focuses on the color whereby certain cell components and/or cell types are actually stained a different color than the original color of the dye.

31. Also, use of the SpectraCube™ system involves the quantitation of euchromatin and heterochromatin and the morphological analysis of cytoplasmic components. It is my

opinion that one of ordinary skill in the art, at the time of the filing of U.S. Patent Application No. 09/306,662, would understand that this description does not describe or suggest utilizing the metachromatic properties of a dye to diagnose cancer as recited in the pending claims.

32. Furthermore, Example 8 of the Cabib et al. patent describes the staining of a cervical smear with haematoxylin-eosin for aiding in the diagnostic pathology as analyzed by a transmission microscopy RGB image. Since haematoxylin stains acidic structures and eosin stains basic structures, the stains in this example are simply being used as contrast agents in order to quantitate various cellular structures to aid in the diagnosis.

33. It is my opinion that one skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teaching of Cabib et al. for the reasons discussed below.

34. For instance, Cabib et al. describes the use of a stain composition that includes two dyes - eosin Y and azure B. There is no suggestion that a metachromatic shift is being used to diagnose dysplasia, pre-cancer or cancer, especially since a non-metachromatic dye (eosin Y) is being used and because Cabib et al. teach a method based on contrast rather than metachromasia. In fact, it would not even be possible to utilize a metachromatic shift in the method of Cabib et al. because the combination of the two dyes would result in the presence of multiple colors, thereby interfering with an examination of the change in color of the single metachromatic dye. Since the method of Cabib et al. measures two colors from two different dyes rather than examining differential color staining from a single metachromatic dye, there is no description or suggestion of the present invention.

35. The Examiner also asserts that the term "metachromasia" is "no more than a fanciful way of describing the change in absorption or transmission spectrum of a dye that occurs after staining two different types of tissues or cells." The Examiner further states that the "'metachromatic shift of the dye' to which the claims refer, thus, is interpreted to denote the change that is observed in the transmission spectrum of a dye after staining a particular tissue or cell relative to that which was previously observed after staining another tissue or cell, or relative to a composite of transmission spectra observed after staining a library of tissues or cells. The change in the absorption or transmission spectra of a dye, or the metachromatic shift is an inherent property of the dye." Therefore, the Examiner asserts that there is "no manipulative difference between the steps practiced in performing the prior art's disclosed methods for diagnosing cancer." The Examiner also concludes that "in practicing the method of the prior art, the artisan necessarily determined the metachromatic shift of the dye that was used to stain the tissue or cells." I disagree with this characterization of a "metachromatic shift" for the reasons discussed below.

36. First, the present invention can be distinguished from the Examiner's definition of metachromasia in that the Examiner asserts that the "metachromatic shift of the dye" is interpreted to denote the change that is observed in the transmission spectrum of a dye after staining a particular tissue or cell. By contrast, the methods of the present invention measure the reflectance spectrum rather than the transmission spectrum. In addition, the Examiner fails to define exactly what this "change" in the transmission spectrum actually is or how it is to be measured. By contrast, the present invention quantifies and compares the observed metachromatic shifts.

37. Furthermore, the Examiner also asserts that the metachromatic shift is an inherent property of the dye. In actuality, the metachromatic shift is not an inherent property of the dye but is a property of the interaction between the dye and the tissue or cell that is being stained.

38. In addition, as explained above, Cabib et al. never uses the term metachromatic shift or even suggests the utilization of the metachromatic interaction between the dyes and the stained samples. Furthermore, Cabib et al. provides no description of a metachromatic shift and provides no suggestion of quantifying the degree of such a shift. The technology disclosed in Cabib et al. does have the ability to measure the metachromatic shifts of dyes; however, the fact that these shifts were not measured suggests that Cabib et al. did not appreciate the potential of using metachromasia in making diagnoses. The additional fact that the Cabib et al. reference is extremely broad and includes a number of applications of morphometric spectral imaging, without including any reference to the use of metachromatic shifts, further suggests to me that Cabib et al. did not recognize the potential use of these shifts in diagnoses.

39. Therefore, it is my opinion that Cabib et al., as would be understood by the person of ordinary skill in the art at the time of the filing of U.S. Patent Application No. 09/306,662, does not describe or suggest, either expressly or inherently, the method as recited in the claims of the pending application for the reasons discussed above.

40. In the Office Action of June 8, 2004, the Examiner has also rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 103(a) as being obvious over U.S. Patent No. 5,784,162 to Cabib et al., in view of Tuite et al. (*Journal of Photochem. and Photobiol. B: Biol.*, 21: 103-124, 1993), as evidenced by Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al.

(*Photochemistry and Photobiology*, 55: 515-522, 1992). The Examiner relies on Cabib et al. for describing methods of using a combination of dyes comprising methylene blue. However, the Examiner admits that Cabib et al. does not teach the use of toluidine blue O. The Examiner also relies on Tuite et al. for describing that toluidine blue O can selectively stain tumor cells and that it is generally non-toxic to normal cells. The Examiner concludes that it would be obvious to have used toluidine blue O in the methods of Cabib et al. because both methylene blue and toluidine blue O had been characterized as non-toxic and are known to selectively stain cancer cells. The Examiner further asserts that one of ordinary skill in the art would have been motivated to use toluidine blue in the methods of Cabib et al. to confirm the results of analyses in which methylene blue had been used.

41. I have already stated my opinion with regard to the Cabib et al. reference above. It is also my opinion that Tuite et al. discuss photochemical interactions of methylene blue and analogues with DNA and other biological substrates. Specifically, methylene blue, Azure B, Azure A, Azure C, thionine and toluidine blue O are discussed. Because all of these dyes are non-toxic and can selectively stain cancer cells, it is my opinion that one skilled in the art would not be motivated to select toluidine blue O to confirm the results of his or her analysis as asserted by the Examiner.

42. Furthermore, as described above, a metachromatic shift can only be detected when a single metachromatic dye is used and cannot be detected with a combination of dyes. However, Cabib et al. never describes or suggests the use of methylene blue by itself. The only mention of a methylene blue related dye is Cabib et al.'s description of the Romanowsky-Giemsa

and Haematoxylin-Eosin staining techniques, which utilize a *combination* of Azure-B and Eosin Y. Therefore, since Cabib et al. never describes or suggests the use of methylene blue by itself, no metachromatic shift could have been observed using the methods of Cabib et al. and it would not have been obvious to use this dye by itself in order to observe such a metachromatic shift.

43. Furthermore, it is my opinion that even if one of skill in the art were to select toluidine blue O for use in the methods of Cabib et al., it would simply be used as a contrast agent. For the reasons discussed above, such use as a contrast agent would be different from the methods of the present invention which compare the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells.

44. It is also my opinion that one of skill in the art would recognize that the Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al. (*Photochemistry and Photobiology*, 55: 515-522, 1992) references do not add any additional disclosure that describes or suggests the methods of the present invention.

45. Therefore, it is my opinion that the person of ordinary skill in the art would understand that there is no description or suggestion in any of these combined references of the method recited in the pending application for the reasons discussed above.

46. I declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or

imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.


Michael R. Hamblin, Ph.D.

Signed in MGH, Boston, MA (location)

this 7 day of OCT, 2004.

U.S.S.N. 09/306,662

Declaration of Michael R. Hamblin

Page 20 of 20

ATTACHMENT A

Curriculum Vitae of Michael R. Hamblin, Ph.D.

PART I: GENERAL INFORMATION*Date Prepared* 8/1/04**Name:** Michael R. Hamblin**Office Address:** Massachusetts General Hospital
Wellman Laboratories of Photomedicine
314B Bartlett Bldg., 40 Blossom Street
Boston, MA 02114**E-mail:** hamblin@helix.mgh.harvard.edu **FAX:** 617-726-8566**Home Address:** 350 Revere Beach Boulevard, Revere, MA 02151**Place of Birth:** Tynemouth, Northumberland, U.K.**Education:**

1970	B.Sc. Hons. Exeter University, U.K. Chemistry
1972	M.Sc. University of Kent at Canterbury, U.K. Enzyme Chemistry
1977	Ph.D. Trent Polytechnic, U.K. Synthetic Organic Chemistry

Postdoctoral Training:

1976-1978	Postdoctoral Fellow, Dept of Chemistry, New University of Ulster, U.K.
1978-1979	Postdoctoral Fellow, Dept of Chemistry, University of Edinburgh, U.K.
1979-1982	Postdoctoral Fellow, Dept of Chemistry, Heriot-Watt University, U.K.

Academic Appointments:

1982-1984	Research Fellow, Dept of Biochemistry, University of Cambridge, U.K.
1984-1987	Research Associate, Dept of Chemistry, Leicester University, U.K.
1990-1994	Cancer Research Campaign Research Fellow, University of Dundee U.K.

- 1994-1997 Instructor, Wellman Laboratories of Photomedicine,
 Department of Dermatology, Harvard Medical School
- 1997- Assistant Professor of Dermatology, Wellman Laboratories
 of Photomedicine, Department of Dermatology, Harvard
 Medical School

Hospital Appointments:

- 1990-1994 Associate in Surgery, Ninewells Hospital and Medical
 School, Dundee, U.K.
- 1994- Assistant in Chemistry, Department of Dermatology,
 Massachusetts General Hospital

Major Administrative Responsibilities:

- 1999- Member: Wellman Laboratories of Photomedicine Education Committee
- 2000-2001 Member: Wellman Laboratories of Photomedicine Cell Biology Faculty Search
 Committee
- 2000- Member: Massachusetts General Hospital Subcommittee on Research Animal
 Care (IACUC)
- 2000-2003 Director: Wellman Laboratories Fall Tutorial Series
- 2002- Member Wellman Laboratories of Photomedicine Finance Committee
- 2003- Joint Chair Wellman-MIT Lester-Wolfe semi annual symposium
- 2004 Member, Wellman Center Space committee
- 2004 Member Center for Photomedicine Faculty Search Committees

Industrial Experience:

- 1987-1990 Managing Director, Columbine Ltd., Brighton, U.K.

Professional Societies:

- 1994 American Society of Photobiology, Member
- 1997 European Society for Photobiology, Member
- 2003 American Association for Cancer Research, Member
- 2004 American Society for Microbiology, Member

Peer reviewing manuscripts for the following Journals:

- Journal of Photochemistry and Photobiology. B: Biology
 Photochemistry and Photobiology

Lasers in Surgery and Medicine
Journal of Biomedical Optics
International Journal of Radiation Biology
British Journal of Cancer
IEEE Journal of Selected Topics in Quantum Electronics
Applied Optics
Cancer Research
Journal of Antimicrobial Chemotherapy
Journal of Pharmacology and Experimental Therapeutics
Biochimica et Biophysica Acta
International Journal of Cancer
Antimicrobial Agents and Chemotherapy
Advanced Drug Delivery Reviews
Archives of Biochemistry and Biophysics
Bioorganic Chemistry
Biochemical Pharmacology
Optics Letters
Infection and Immunity
Photodiagnosis and Photodynamic Therapy

Peer reviewing grants for the following awarding bodies:

Ligue Suisse Contre le Cancer, Bern, Switzerland
National Cancer Institute of Canada, Toronto, ON, Canada
U.S. Civilian Research and Development Foundation (CRDF-ISTC)
Association for International Cancer Research, St Andrews, UK
Wellcome Trust, London, UK

PART II: RESEARCH AND TEACHING CONTRIBUTIONS

A. Major Research Interests:

Photodynamic therapy (PDT) is a relatively new and exciting approach for treating cancers and other diseases. Photosensitizers (PS) are administered systemically, locally or topically and accumulate in the tumor or other lesion. Illumination with visible (usually red light, frequently from a laser) excites the sensitizer, which in the presence of oxygen, produces cytotoxic or stimulatory effects. My particular area of interest is in the study of macromolecular conjugates of PS as targeting agents. Large molecules have very different biodistribution and pharmacokinetics compared to the small molecules that are generally used as PS. This strategy has been applied to devise novel methods of treating cancer, infections and heart disease.

Cancer. Conjugates between PS and modified albumin can be readily and specifically taken up by macrophages via the high capacity scavenger receptor. Tumor associated macrophages (TAMs) can be selectively killed or modified by the appropriate PDT regimen. It has become apparent in recent years that TAMs are partly responsible for the growth, invasion and metastasis of tumors and are therefore a valid target for cancer therapy. This may be accomplished by a binary approach in which the PS-conjugate targets the macrophages and the spatial confinement of the light delivery ensures that only TAMs (bad) are killed and other macrophages (good) are spared. In addition, I have recently begun to concentrate on more closely defining the anti-tumor effects of PDT by using various syngeneic mouse tumors in immunocompetent mice. Research goals here include the identification of specific PDT regimens, and immunostimulants (toll-like receptor ligands) to maximize the generation of anti-tumor immunity.

PDT for localized infections. Polycationic chlorin e6 conjugates with a pronounced positive charge are able to effectively target bacteria (both Gram (+) and Gram (-)) for photodestruction. This is thought to be mediated by the structure of the polycationic carrier being able to disrupt the outer-membrane permeability barrier typical of Gram (-) bacteria, while Gram (+) species are very susceptible to PDT. Considerable data on the structure-function relationships of these conjugates and their efficiency in photodynamic inactivation of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* have been accumulated, and in optimum doses can give six logs of killing. Multi-antibiotic resistant bacteria can be killed as easily as naive strains. I have developed several mouse models of infections using pathogenic bacteria transfected with the gene complex coding for luciferase and its substrates and a sensitive photon-counting camera to image the light emitted from the animals in real time to follow the progress of the infection. These now comprise excisional wounds, soft tissue infections in neutropenic and immunocompetant mice, chronic abscesses, burns and bladder infections. Topical or interstitial administration of conjugates, followed by illumination eradicates the infection, and in the case of pathogenic strains, save the lives of the mice which would otherwise die of systemic sepsis. The treatment does not damage host tissue as shown by the wound healing response being as good as or better than control wounds treated by alternative antimicrobial therapies.

Diagnosis and therapy of vulnerable atherosclerotic plaque (VP). It is now accepted that the non-stenotic highly inflamed atherosclerotic plaque with a thin collagen

cap in the coronary arteries is vulnerable to rupture, frequently causing a massive coronary thrombosis and sudden death. Since most patients with vulnerable plaque have no prior symptoms of heart disease, there is increasing interest in technologies to both detect and treat VP. It is clear that the most important cellular component and causative agent within VP is the activated macrophage, which is responsible for collagen cap degradation by secreting matrix metalloproteinases thus increasing likelihood of rupture. Since the scavenger-targeted conjugates (modified serum albumin, see above) show a high degree of selectivity for macrophages, they are an attractive targeting vehicle for delivering fluorescent dyes to VP allowing intravascular fluorescence diagnosis, and delivering photoactive dyes allowing photodynamic stabilization of VP by increasing the fibrous cap and reducing the inflammatory macrophages.

B. Research Funding Information:

Past

1994-1996	NIH/R01 - Co-Investigator (T. Hasan, PI) Experimental Photoimmunotherapy of Ovarian Cancer.
1996-1998	Periodontix Inc. - Co-Investigator (T. Hasan, PI) Photodynamic therapy of periodontitis.
1999-2005	Department of Defense - Co-Investigator (JA Parrish, PI) Program to Develop Biomedical Applications of the Free Electron Laser Photoimmunotherapy for the local control of sepsis.
1999-2006	Department of Defense - Co-Investigator (JA Parrish, PI) Program to Develop Biomedical Applications of the Free Electron Macrophage targeted photodynamic regulation of wound healing.
1997-2000	NIH/R01 - Co-Investigator (T. Hasan, PI) Experimental Photoimmunotherapy of Ovarian Cancer. (competing continuation)
2003-2004	Department of Defense - Surgical Laser Applications from MFEL studies - Project leader (JA Parrish, PI) Photodynamic destruction of tissue invasive pathogens in animal burn models.
2001-2002	CIMIT New Concept Award - Principal Investigator Macrophage-targeted PDT for diagnosis and therapy of vulnerable plaque. \$25,000 direct
2002-2003	DAMD 17-02-2-0006 - CIMIT Proof-of Principle Award - Principal Investigator Macrophage-targeted PDT for diagnosis and therapy of vulnerable plaque. \$75,000 direct

2001-2003 Seedling Enterprises - Principal Investigator. Light-mediated killing of *Helicobacter pylori*: an in vitro and ex vivo study. \$102,000 direct

Current

2003-2005 N00014-94-1-0927 Department of Defense - Program to Develop Biomedical Applications of the Free Electron Laser - Project leader (JA Parrish, PI)
Photodynamic inactivation of pathogenic bacteria in contaminated wounds.

2001-2006 NIH/1 PO1 CA84203-01- Core Director (Program Director T. Hasan)
In vivo PDT: Animals, Dosimetry and Statistics Core. \$924,115 direct

2002-2005 NIH/R01 CA/AI838801-A2- Principal Investigator
Macrophage-targeted PDT. \$435,000 direct

2002-2007 NIH/BRP- 1R01 EY14106-01 - Project Leader (CP Lin, PI)
Live microscopy and cytometry in vascular biology. \$506,704 direct

2002-2005 NIH/R01 - Investigator (NS Soukos, PI)
Photosensitization of oral bacteria. 5% effort (\$450,000 direct)

2003-2006 CIMIT New concept Award - Principal Investigator
Macrophage-Targeted Fluorescent Detection of vulnerable plaque. \$25,000 direct

2003-2007 NIH/1R01 AI050875-01A1 - Principal Investigator -Photodynamic Therapy for the Treatment of Localized Infections. \$700,000 direct

2003-2004 NIH/SBIR (T. Wharton PI). Sub-contract PI
Novel Nanostructures for Photodynamic Therapy. \$62,000 direct

2003-2004 LumeRx Corp - Principal Investigator. Phototherapy for *Helicobacter pylori* infection. \$102,000 direct,

2004 NIH/SBIR (H. Gali PI)- Sub-contract PI
Receptor-Targeted Photosensitizers for PDT of Cancers. \$16,000 direct

2004-2005 HemCon Inc - Principal Investigator. Antimicrobial effects of HemCon bandage. \$42,000 direct

Pending

- 2004 NIH/NIAID R01, Principal investigator, PDT for Buruli Ulcer Disease, \$600,000 direct
- 2004 NIH/SBIR (T Wharton PI)- subcontract PI, Photodynamic Blood Product Decontamination, \$69,620 direct
- 2004 NIH/SBIR (H Gali, PI) – subcontract PI, Novel nanoparticles for targeted photothermal therapy. \$65,240 direct
- 2004 NIH/NHLBI R01, Principal investigator, Targeted PDT for vulnerable atherosclerotic plaque. \$1,250,000 direct.
- 2004 NIH/SBIR (G Burke, PI) – subcontract PI, Fluorescence detection of vulnerable plaques in vivo. \$33,240 direct

C. Teaching Experience:**1. Local Contributions:**Undergraduate and Graduate Courses:

- 1970-1971 Chemistry Master, St. Hughs High School, Birkenhead, U.K.
Taught chemistry to GCE 'O' level (full time teaching)
- 1972-1976 Research Assistant Demonstrator, Trent Polytechnic.
Taught lecture course in first year organic chemistry to B.Sc. Hons Applied Science students (approx 60 students, 12 hours/year)
Jointly ran laboratory classes in organic chemistry for all four years of B.Sc. Hons Applied Science course (120 hours/year).
Conducted tutorials in organic chemistry for all four years of B.Sc. Hons Applied Science course (80 hours/year)
- 1982-1984 Supervisor, University of Cambridge.
Conducted supervisions in organic chemistry for Trinity and Churchill colleges (50 hours/year).
- 1984-1987 Demonstrator, Leicester University
Jointly conducted laboratory classes in organic chemistry for all three years of B.Sc. Hons Chemistry course (100 hours/year).

- 1994- Delivered tutorial lectures in Wellman Laboratories
Photomedicine Lecture Series
- 1997 Course on Photodynamic Therapy and Fluorescence
Diagnosis for the Electro-Optics Center, Tufts University,
Medford MA.

Advisees, Trainees:

- 1976-1978 One post-graduate student
- 1979-1982 Two post-graduate students
- 1982-1984 Two post-graduate students
- 1984-1987 Two post-graduate students
- 1990-1994 One post-graduate student, two post-doctoral fellows
one technician
- 1994- Eighteen undergraduate students,
- | | | |
|-----------------|-----------|--------------------------|
| Imran Rizvi | 1994-1997 | Wellman Laboratories |
| Jaimie Miller | 1994-1997 | Wellman Laboratories |
| Pradeep Penta | 1997 | MIT |
| Naveen Murthy | 1997 | GlycoGenesis Inc |
| Yeshaya Koblick | 1999 | Tufts University |
| David Adam | 2000 | University of Toronto |
| Zaraq Khan | 2001 | Aga Khan Medical College |
| Azadeh Shirazi | 2002 | University of Kentucky |
| Aamir Ahmad | 2002 | Aga Khan Medical College |
| Maria Maqsood | 2002 | Aga Khan Medical College |
| Maleha Khan | 2002 | Aga Khan Medical College |
| Imran Khan | 2003 | Aga Khan Medical College |
| Umber Khan | 2003 | Aga Khan Medical College |
| Miram Afridi | 2003 | Aga Khan Medical College |
| Madiha Kamal | 2003 | Aga Khan Medical College |
| Sumbul | 2004 | Aga Khan Medical College |
| Hina | 2004 | Aga Khan Medical College |

Ibrahim Rizqi

2004

Aga Khan Medical College

Nineteen post-doctoral fellows,

Tetsuo Momma MD	1994-1996	Tokyo University Hospital
Nikolaos Soukos DDS, PhD	1994-2000	Forsyth Institute, Boston
Marco Del Governatore MD	1994-1996	University of Bologna, Italy
Linda Duska MD	1995-1997	Massachusetts General Hospital
Frank Konig MD	1996-1998	Charite Hospital, Berlin
Misbah Huzaira MD	1997	Massachusetts General Hospital
Tetsuya Kodama PhD	1998-2000	Imperial College, London
Touqir Zahra MD	2000-2001	Newton Wellesley Hospital
Zihua Wang, Ph.D.	2002	Boston Biotech
Faten Gad, M.D.	2002-2004	Wellman Laboratories
Qingde Liu, M.D., Ph.D.	2002-	Wellman Laboratories
Ana Patricia Castano, M.D.	2002-	Wellman Laboratories
Xun Sun, Ph.D.	2002	Massachusetts Eye and Ear Infirmary
Andrea Bell, Ph.D.	2002	Leeds University, UK
George Tegos, Ph.D.	2003-	Wellman Laboratories
Changming Yang	2003-	Wellman Laboratories
Florencia Anatelli	2003-	Wellman Laboratories
Dennisse Arcila Lopez	2004-	Wellman Center
Mohammed Yawar Yakoob	2004-	Wellman Center

Eleven technicians.

Jaimie Miller BS	1997-1999	Columbia University
Imran Rizvi, BS	1997-2002	Georgetown University
Michael Bamberg BS	1994-1999	Ilex Oncology
David O'Donnell BS	1998-2000	Fleet Boston Financial
Atosa Ahmadi BS	2000-2001	Suffolk University
Jeremy Stern, BS	2001-2003	Law School
Samuel J Whitaker, BS	2002	Philadelphia
Stephanie Chirico, BS	2002-2004	Providence RI
Jennifer Viveiros, BS	2002-	Wellman Laboratories
Tatiana Demidova, MS	2002-	Wellman Laboratories
Victoria Hamrahi, BS	2002	Shriners Burn Institute

2. Regional, national, or international contributions

- 1994 Photochemical Targeting and Medical Applications
IEEE Lasers and Electro-Optics Society LEOS 94, Boston, MA.
- 1997 Light mediated modulation of wound healing, in the Plenary Session 6: Laser Tissue Interactions and Wound Healing, of the Twentieth Biennial Cornea Research Conference, Massachusetts Eye and Ear Infirmary, Boston, MA.
- 1999 Photodynamic antisepsis, ONR Contractors Meeting, Institute of Surgical Research, Fort Sam Houston, San Antonio, TX
- 2000 Photodynamic therapy: mechanisms, targeting, and applications, Duke Medical Free Electron Laser Laboratory Duke University, Durham, NC
- 2000 Photodynamic inactivation of pathogenic bacteria in contaminated wounds, MFEL-ONR contractors meeting, Newport Beach, CA
- 2000 Use of luminescent bacteria to demonstrate photodynamic inactivation in contaminated wounds, Dept of Pediatrics, Stanford University School of Medicine, Stanford, CA
- 2002 Targeted photosensitizer conjugates: specific and versatile? Photodynamic Therapy Center, Roswell Park Cancer Center, Buffalo, NY
- 2002 Scavenger receptor-targeted photodynamic therapy of J774 tumors in mice: tumor response and concomitant immunity. BioS 2002 Biomedical Optics, SPIE Photonics West, Laser Tissue Interaction XIII: Photochemical, Photothermal, and Photomechanical. San Jose, CA
- 2002 Use of genetically engineered bioluminescent bacteria to develop animal models of localized infections suitable for photodynamic therapy. IQEC/LAT2002 Conference on Lasers, Applications and Technologies, Moscow, Russia
- 2002 Degree of substitution of chlorine6 conjugated to charged poly-L-lysine chains affects their cellular uptake, localization and phototoxicity. Saratov International Workshop on Biophotonics – SIWB02 Saratov, Russia
- 2002 Scavenger receptor-targeted photodynamic therapy for diagnosis of vulnerable atherosclerotic plaques. Saratov International Workshop on Biophotonics – SIWB02 Saratov, Russia
- 2003 Photodynamic therapy of mouse tumors; local control and anti-tumor immunity. (Invited Lecture). BioS 2003 Biomedical Optics, SPIE Photonics West, Laser Tissue Interaction XIV: Photochemical, Photothermal, Photomechanical, San Jose, CA

- 2003 Induction of anti-tumor immunity by photodynamic therapy of mouse tumors. (Invited Lecture). 31st Annual Meeting of American Society for Photobiology, Baltimore, MD
- 2003 Invited Chair - Contributed papers session 1, 31st Annual Meeting of American Society for Photobiology, Baltimore, MD
- 2004 Induction of anti-tumor immunity by photodynamic therapy of mouse tumors. (Invited Lecture). BioS 2004 Biomedical Optics, SPIE Photonics West, Laser Tissue Interaction XV: Photochemical, Photothermal, Photomechanical, San Jose, CA
- 2004 Invited chair - Session 6, Optical Techniques for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XIII, BioS 2004 Biomedical Optics, SPIE Photonics West, San Jose, CA
- 2004 Invited chair - Session 2, Laser Tissue Interaction XV: Photochemical Photothermal, Photomechanical, BioS 2004 Biomedical Optics, SPIE Photonics West, San Jose, CA
- 2005 Antimicrobial photodynamic therapy. Centre for Photobiology and Photodynamic Therapy, School of Biochemistry and Molecular Biology, University of Leeds, United Kingdom.
- 2004 Anti-tumor immunity generated by photodynamic therapy in a metastatic murine tumor. 32nd Annual Meeting of American Society for Photobiology, Seattle WA.
- 2004 Invited Chair. Session III: Light Activated Tissue Repair and Regeneration Using Exogenous Chromophores. Light Activated Tissue Regeneration and Therapy I, Ohana Keauhou Beach Resort, Kona Coast, Hawaii.
- 2004 Photodynamic modulation of wound healing and inhibition of tissue degradation. Light Activated Tissue Regeneration and Therapy I, Ohana Keauhou Beach Resort, Kona Coast, Hawaii.
- 2004 Photodynamic tissue repair and healing. Light Activated Tissue Regeneration and Therapy I, Ohana Keauhou Beach Resort, Kona Coast, Hawaii.
- 2004 Use of genetically engineered bioluminescent bacteria to develop animal models of localized infections suitable for photodynamic therapy. Medical Division, 3M Corporation, St-Paul, MN.

PART III: BIBLIOGRAPHY***Original Reports:***

1. Coutts IG, **Hamblin MR**. Synthesis of N,N-diaryltoluene-4-sulphonamides. *J Chem Soc Perkin I* 1975:2445-46.
2. Coutts IG, **Hamblin MR**. An unusual reaction of methylmagnesium iodide with cyclohexadienones. *J Chem Soc Chem Commun* 1976:58-59.
3. Coutts IG, **Hamblin MR**, Tinley EJ. The enzymatic oxidation of phenolic tetrahydroisoquinoline-1-carboxylic acids. *J Chem Soc Perkin I* 1979:2744-50.
4. Grundon MF, **Hamblin MR**, Harrison DM. Biosynthesis of Aromatic Isoprenoids Part 5: The preparation of 1-(3,3-dimethylallyl)-L-tryptophan and cyclo-L-alanyl tryptophan and their non-incorporation into echinulin. *J Chem Soc Perkin I* 1980:1294-98
5. Buchanan JG, **Hamblin MR**, Sood GR, Wightman RH. The biosynthesis of pyrazofurin and formycin. *J Chem Soc Chem Commun* 1980:917-18.
6. Coutts IG, **Hamblin MR**. Synthesis of spiroheterocycles by oxidative coupling of phenolic sulphonamides. *J Chem Soc Chem Commun* 1980:949-50.
7. Coutts IG, **Hamblin MR**. Spirodienones Part 2: The synthesis of some heterocyclic spirodienones by phenolic coupling. *J Chem Soc Perkin I* 1981:493-97.
8. Buchanan JG, **Hamblin MR**, Kumar A, Wightman RH. The biosynthesis of showdomycin – Studies with stable isotopes and the determination of principal precursors. *J Chem Soc Chem Commun* 1984:1515-17.
9. **Hamblin MR**, Potter BV. E. coli Ada regulatory protein repairs the SP diastereoisomer of alkylated DNA. *FEBS Lett* 1985;189(2):315-17.

10. **Hamblin MR**, Cummins JH, Potter BV. Mung bean nuclease catalyzes DNA cleavage with inversion of configuration at phosphorous. *Biochem Soc Trans* 1986;14:899-900.
11. **Hamblin MR**, Potter BV, Gigg R. Bisphosphorylation of a vic-diol using a phosphite chemistry approach. Synthesis of myo-inositol 4,5-bisphosphate. *J Chem Soc Chem Commun* 1987:626-27.
12. **Hamblin MR**, Flora JS, Potter BV. Myo-Inositol phosphorothioates, phosphatase-resistant analogues of myo-inositol phosphates. Synthesis of DL-myo-inositol 1,4-bisphosphate and DL-myo-inositol 1,4-bisphosphorothioate. *Biochem J* 1987;246(3):771-74.
13. **Hamblin MR**, Potter BV, Gigg R. Synthesis of myo-inositol phosphates and analogues using a phosphite chemistry approach. *Biochem Soc Trans* 1987;15:415-16.
14. **Hamblin MR**, Cummins JH, Potter BV. Mung bean (*Phaseolus aureus*) nuclease. A mechanistic investigation of the DNA-cleavage reaction using a dinucleoside phosphorothioate. *Biochem J* 1987;241(3):827-33.
15. **Hamblin MR**, Newman EL. Photosensitizer targeting in photodynamic therapy. I. Conjugates of haematoporphyrin with albumin and transferrin. *J Photochem Photobiol B* 1994;26(1):45-56.
16. **Hamblin MR**, Newman EL. Photosensitizer targeting in photodynamic therapy. II. Conjugates of haematoporphyrin with serum lipoproteins. *J Photochem Photobiol B* 1994;26(2):147-57.
17. Molpus KL, Kato D, **Hamblin MR**, Lilge L, Bamberg M, Hasan T. Intraperitoneal photodynamic therapy of human epithelial ovarian carcinomatosis in a xenograft murine model. *Cancer Res* 1996;56:1075-82.
18. **Hamblin MR**, Miller JL, Hasan T. The effect of charge on the interaction of site-specific photoimmunoconjugates with human ovariancancer cells. *Cancer Res* 1996; 56:5205-10.

19. Duska LR, **Hamblin MR**, Bamberg MP, Hasan T. Biodistribution of charged F(ab')₂ photoimmunoconjugates in a xenograft model of ovarian cancer. *Br J Cancer* 1997;75:837-44. (the first two authors made equal contributions)
20. Soukos NS, **Hamblin MR**, Hasan T. The effect of charge on cellular uptake and phototoxicity of polylysine chlorin_{e6} conjugates. *Photochem Photobiol* 1997;65:723-29. (the first two authors made equal contributions)
21. Momma T, **Hamblin MR**, Hasan T. Hormonal modulation of the accumulation of 5-aminolevulanic acid-induced protoporphyrin and phototoxicity in prostate cancer cells. *Int J Cancer* 1997;72:1062-69.
22. Soukos NS, Ximenez-Fyvie LA, **Hamblin MR**, Socransky SS, Hasan T. Targeted antibacterial photochemotherapy. *Antimicrob Agents Chemother* 1998;42:2595-01.
23. **Hamblin MR**, Bamberg MP, Miller JL, Hasan T. Cationic photoimmunoconjugates between monoclonal antibodies and hematoporphyrin: selective photodestruction of ovarian cancer cells. *Applied Optics*, 1998;37:7184-92.
24. Momma,T, **Hamblin MR**, Wu HC, Hasan T. Photodynamic therapy of orthotopic prostate cancer with benzoporphyrin derivative: local control and distant metastasis. *Cancer Res*, 1998;58:5425-5431.
25. **Hamblin MR**, Rajadhyaksha M, Momma T, Soukos NS, Hasan T. *In vivo* fluorescence imaging of the transport of charged chlorin_{e6} conjugates in a rat orthotopic prostate tumor. *Br J Cancer* 1999;81:261-68.
26. Duska LR, **Hamblin MR**, Miller JL, Hasan T. Photoimmunotherapy in combination with cisplatin administration for the treatment of advanced epithelial ovarian cancer. *J Natl Cancer Inst* 1999;91:1557-63.
27. Khadem J, Veloso Jr. AA, Tolentino F, Hasan T, **Hamblin MR**. Photodynamic tissue welding with chlorin(e6) protein conjugates. *Invest Ophthal Vis Sci* 1999;40:3132-37.

28. Del Governatore M, **Hamblin MR**, Piccinini EE, Ugolini G, Hasan T. Targeted photodestruction of human colon cancer cells using charged 17.1a chlorin_{e6} immunoconjugates. *Br J Cancer* 2000;82:56-64.
29. Molpus KL, **Hamblin MR**, Rizvi I, Hasan T. Intraperitoneal photoimmunotherapy of ovarian carcinoma xenografts in nude mice using charged photoimmunoconjugates. *Gynecol Oncol* 2000;76:397-404.
30. Del Governatore M, **Hamblin MR**, Shea CR, Rizvi I, Molpus KG, Tanabe K, Hasan T. Experimental photoimmunotherapy of hepatic metastases of colorectal cancer with a 17.1A chlorin_{e6} immunoconjugate. *Cancer Res*, 2000;60:4200-05.
31. Kodama T, **Hamblin MR**, Doukas, AG. Cytoplasmic molecular delivery with shock waves: importance of impulse. *Biophysical J*, 2000;79:1821-32.
32. **Hamblin MR**, Miller, JL, Ortel B. Scavenger-receptor targeted photodynamic therapy. *Photochem Photobiol* 2000;72:533-40.
33. **Hamblin MR**, Del Governatore M, Rizvi I, Hasan T. Biodistribution of charged 17.1a photoimmunoconjugates in a murine model of hepatic metastasis of colorectal cancer. *Br J Cancer* 2000;83:1544-51.
34. Soukos NS, **Hamblin MR**, Keel S, Fabian RL, Deutsch TF, Hasan T. Epidermal growth factor receptor targeted immunophotodiagnosis and photoimmunotherapy of oral precancer *in vivo*. *Cancer Res*, 2001;61:4490-96.
35. **Hamblin MR**, Miller JL, Rizvi I, Ortel B, Maytin EV, Hasan T. Pegylation of a chlorin_{e6} polymer conjugate increases tumor targeting of photosensitizer. *Cancer Res* 2001;61:7155-62.
36. **Hamblin MR**, O'Donnell DA, Murthy N, Contag CH, Hasan T. Rapid control of wound infections by targeted photodynamic therapy monitored by *in vivo* bioluminescence imaging. *Photochem Photobiol* 2002;75:51-57.
37. Kodama T, Doukas, AG, **Hamblin MR**. Shock wave-mediated molecular delivery into cells. *Biochem Biophys Acta* 2002;1542:186-94.

38. **Hamblin MR**, O'Donnell DA, Murthy N, Rajagopalan K, Michaud N, Sherwood ME, Hasan T. Polycationic photosensitizer conjugates: photodynamic inactivation of bacteria depends on conjugate structure and Gram classification. *J Antimicrob Chemother* 2002;49:941-51.
39. **Hamblin MR**, Miller JL, Rizvi I, Ortel B. Degree of substitution of chlorin(e6) conjugated to charged poly-L-lysine chains affects their cellular uptake, localization and phototoxicity towards macrophages and cancer cells. *J X-ray Science and Technology* 2002;10:139-52.
40. Kodama T, Doukas, AG, **Hamblin MR**. Delivery of ribosome-inactivating protein toxin into cancer cells with shock waves. *Cancer Lett* 2003;189:69-75.
41. **Hamblin MR**, Zahra T, Contag CH, McManus AT, and Hasan T. Optical monitoring and treatment of potentially lethal wound infections *in vivo*. *J Infect Dis* 2003;187:1717-25.
42. **Hamblin MR**, Miller JL, Rizvi I, Loew HG, and Hasan T. Pegylation of charged polymer-photosensitizer conjugates: effects on photodynamic efficacy. *Br J Cancer*, 89: 937-943, 2003
43. Gad F, Zahra T, Francis KP, Hasan T, and **Hamblin MR**. Targeted photodynamic therapy of established soft-tissue infections in mice. *Photochem Photobiol Sci*, 2004; 3: 451 – 458
44. **Hamblin MR** and Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem Photobiol Sci*, 2004; 3: 436 – 450
45. Gad F, Zahra T, Hasan T, and **Hamblin MR**. Photodynamic inactivation of Gram-positive pathogenic bacteria: effect of growth phase and extracellular slime. *Antimicrob Agents Chemother*, 2004; 48:2173-2178.
46. Shi L, Takahashi K, Dundee J, Shahroor-Karni S, Thiel S, Jensenius JC, Gad F, **Hamblin MR**, Sastry KN, Ezekovitz RA. Mannose-binding lectin deficit mice are

- susceptible to infection with *Staphylococcus aureus*. J Exp Med., 2004, 199:1379-1390
47. Demidova TN and **Hamblin MR**, Macrophage targeted photodynamic therapy. Int J Immunopathol Pharmacol. 2004 17(2):117-126
48. Demidova TN and **Hamblin MR**, Photodynamic therapy targeted to pathogens. Int J Immunopathol Pharmacol, *in press*, 2004
49. Khadem JJ, Martino M, Anatelli F, Dana MR, **Hamblin MR**. Healing of perforating rat corneal incisions closed with photodynamic laser-activated tissue glue. Lasers Surg Med, *in press*, 2004
50. Liu Q and **Hamblin MR**, Macrophage-targeted photodynamic therapy: scavenger receptor expression and activation state. J Pharmacol Exp Therapeut (*submitted* 2004).
51. Ganz RA, Viveiros J, Ahmad A, Ahmadi A, Khalil A, Tolckoff MJ, Nishioka NS, **Hamblin MR**. *Helicobacter pylori* in patients can be killed by visible light. Gastrointestinal Endoscopy, (*submitted*, 2004)
52. Demidova TN and **Hamblin MR**, Microbial photoinactivation: effect of cell-photosensitizer binding and cell density. (*submitted* 2004).
53. Lambrechts SA, Demidova TN, Aalders MC, Hasan T, **Hamblin MR**, Photodynamic treatment of *Staphylococcus aureus* infected burn wounds in mice. Lasers Surg Med. (*Submitted* 2004)
54. Demidova TN, Gad F, Zahra T, Francis KP, **Hamblin MR**. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. J Photochem Photobiol B, (*Submitted* 2004)
55. **Hamblin MR**, Viveiros J, Yang C, Ganz RA, Tolckoff MJ. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. J Infect Dis, (*Submitted* 2004)

Proceedings of Meetings:

56. **Hamblin MR**, Newman EL. Conjugates between proteins and fluorescent dyes as potential photosensitizers. In: dal Fante M, Spinelli P, Marchesini R, editors. Photodynamic Therapy and Biomedical Lasers. Proceedings of the International Conference on Photodynamic Therapy and Medical Laser Applications; Sep 4-10, 1992 Milan, Italy. Amsterdam: Elsevier; 1992. p. 518-20.
57. Soukos NS, **Hamblin MR**, Deutsch TF, Hasan T. Monoclonal antibody-tagged receptor-targeted contrast agents for detection of cancers. In: Bearman GH; Bornhop DJ; Levenson RM; Editors. Biomarkers and Biological Spectral Imaging, Jan 19-23, 2001, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE; 2001. p. 115-28. 4259
58. **Hamblin MR**, O'Donnell DA, Zahra T, Contag CH, McManus AT, Hasan T. Targeted photodynamic therapy for infected wounds in mice. In: Dougherty TJ editor, Optical methods for tumor treatment and detection: Methods and techniques in Photodynamic therapy XI, Jan 20-24, 2002, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE; 2002. Vol 4612: p.48-58.
59. **Hamblin MR**, O'Donnell DA, Huzaira M, Zahra T. Scavenger receptor-targeted photodynamic therapy of J774 tumors in mice: tumor response and concomitant immunity. (Invited paper). In: Jacques SL, Duncan DD, Kirkpatrick SJ, Kriete A, editors, Laser Tissue Interaction XIV: Photochemical, Photothermal, and Photomechanical, Jan 25-31, 2002, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE; 2002. Vol 4617: p. 1-10.
60. Castano AP, Gad F, Zahra T, **Hamblin MR**. Specific anti-tumor immune response with photodynamic therapy mediated by benzoporphyrin derivative and chlorin(e6). (Invited paper) In: Jacques SL, Duncan DD, Kirkpatrick SJ, Kriete A, editors, Laser Tissue Interaction XIII: Photochemical, Photothermal, and Photomechanical, Jan 25-31, 2003, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE; 2003, Vol 4612: p 1-9

61. **Hamblin MR**, Tawakol A, Castano AP, Gad F, Zahra T, Ahmadi A, Stern J, Ortel B, Chirico S, Shirazi A, Syed S Muller JE. Macrophage-targeted photodynamic detection of vulnerable atherosclerotic plaque. In: Gregory KW, Whittaker P, Woodburn KW Editors, *Lasers in Surgery: Advanced Characterization, Therapeutics, and Systems*, Jan 25-31 2003, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE; 2003, Vol 4949: p 466-476.
62. Gad F, Zahra T, Hasan T, and **Hamblin MR**. Targeted photodynamic therapy of established soft-tissue infections in mice. In: Kessel D, Editor, *Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy*, Jan 24-29 2004, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE, 2004 *in press*.
63. Castano AP, Liu Q, and **Hamblin MR**. Photodynamic therapy cures green fluorescent protein expressing RIF1 tumors in mice. In: Jaques SL, Roacch WP Editors, *Laser Interaction with Tissue and Cells XV*, Jan 24-29 2004, Bellingham, WA, The International Society for Optical Engineering, Proceedings of SPIE, 2004 *in press*
64. Photons for therapy: Targeted photodynamic therapy for infected and contaminated wounds. **Hamblin MR**, Gad F, Anderson RR, Hasan T. NATO Research and Technology Organisation Symposium: Combat Casualty Care in Ground Based Tactical Situations: Trauma Technology and Emergency Medical Procedures. HFM-109 /RSY organised by the Human Factors and Medicine Panel in cooperation with the US Department of Defense Advanced Technology Applications for Combat Casualty Care Conference. *In press*

Reviews and Book Chapters:

- 1 **Hamblin MR**, Newman EL. New Trends in Photobiology: On the mechanism of the tumour-localising effect in photodynamic therapy. *J Photochem Photobiol B* 1994;23(1):3-8.
- 2 **Hamblin MR**, Hasan T. Advances in photodynamic therapy. *Optics and Photonic News* 1996;7(7):16-21.

- 3 **Hamblin MR**, Ortel B. Future directions: Photosensitizer targeting and new disease indications. In: Hader DM, Jori G, series editors. Comprehensive series in photosciences. Amsterdam, Netherlands: Elsevier Science, BV; 2001. p. 339-66. (Calzavara-Pinton PG, Ortel B, Szeimies RM, volume editors. Photodynamic therapy and fluorescence diagnosis in dermatology; vol. 2.)
- 4 **Hamblin MR**. Covalent photosensitizer conjugates for targeted photodynamic therapy. Trends in Photochem Photobiol 2002;9:1-24.
- 5 **Hamblin MR**. Photodynamic therapy. In: Syrigos KN and Harrington K, editors. Targeted therapy for cancer. Oxford UK: Oxford University Press; 2003. p. 259-72.

Abstracts:

1. **Hamblin MR**, Newman EL. Protein-porphyrin conjugates as photosensitizers. American Society for Photobiology. Chicago, IL; 1993: Photochem Photobiol 1993; 57:89S.
2. **Hamblin MR**, Bamberg M, Hasan T. The preparation of site-specific monoclonal antibody hematoporphyrin conjugates via poly-L-lysine linkers. American Society of Photobiology. Washington, DC; 1995: Photochem Photobiol 1995;61:95S.
3. **Hamblin MR**, Duska LR, Molpus KL, Bamberg MP, Hasan T. Preparation and biodistribution of site-specific immunoconjugates between OC125 anti-ovarian cancer monoclonal antibody and chlorin e_6 using poly-L-lysine linkers of opposite charge in a nude mouse xenograft model. Monoclonal Antibodies and Cancer Therapy - The Next Decade. New York, NY: Cancer Research Institute; 1995:M04.
4. Duska LR, **Hamblin MR**, Bamberg MP, Molpus K, Hasan T. Biodistribution of monoclonal antibody photosensitizer conjugates of opposite charge in a nude mouse xenograft model of ovarian cancer. NEAGO 1995.

5. Molpus KL, **Hamblin MR**, Bamberg M, Hasan T. Intraperitoneal photoimmunotherapy of human ovarian carcinoma in a xenograft model using charged conjugates. NEAGO 1996.
6. **Hamblin MR**, Rajadhyaksha M, Momma T, Hasan T. The effect of size and charge on the transport and localization of photosensitizer conjugates in an orthotopic rat prostate cancer model. American Society for Photobiology. Atlanta, GA; 1996: Photochem Photobiol 1996;63:23S.
7. Del Governatore M, **Hamblin MR**, Hasan T. Targeting hepatic metastases of colorectal cancer by charged photoimmunoconjugates. Twelfth International Conference on Photobiology. Vienna, Austria; 1996.
8. Molpus K, **Hamblin MR**, Rizvi I, Bamberg M, and Hasan T, Intraperitoneal photoimmunotherapy of human ovarian carcinoma in a xenograft model using charged conjugates. Society of Gynecological Oncologists 1997, Gynecol Oncol 64:302,1997.
9. Soukos NS, **Hamblin MR**, Ximenez-Fyvie LA, Socransky SS and Hasan T. Photolysis of oral bacteria by polylysine chlorin e6 conjugates of different charge. Gordon Research Conference on Periodontal Diseases, New England, 1997
10. **Hamblin MR**, Miller JL, Rizvi I, and Hasan T, Pegylated poly-l-lysine chlorin_{e6} conjugates as photosensitizers. American Society for Photobiology, St Louis MO; 1997: Photochem Photobiol 1997; 65: 8S.
11. **Hamblin MR**, Momma T, and Hasan T. Experimental intraoperative photodynamic therapy for prostate cancer. American Society for Photobiology, St Louis MO; 1997: Photochem Photobiol 1997;65:60S.
12. **Hamblin MR**, Miller JL, Rizvi I, and Hasan T. Targeted photosensitization with pegylated poly-l-lysine chlorin e6 conjugates. Europto Series: Photochemotherapy of cancer and other diseases, BIOS '97 San Remo, Italy 1997

13. **Hamblin MR**, Soukos NS, and Hasan T, Selective photoinactivation of gram positive and gram negative bacteria while sparing mammalian cells. European Society for Photobiology, Stresa, Italy; 1997.
14. Soukos NS, **Hamblin MR**, Ximenez-Fyvie LA,, Socransky SS, and Hasan T. Photolysis of oral bacteria by polylysine chlorin e6 conjugates of different charge. Gordon Research Conference on Periodontal Diseases, New Hampshire, 1997.
15. **Hamblin MR**, Duska LR, del Governatore M, Momma T, and Hasan T, Tumor targeting and tissue transport of charged poly-l-lysine chlorine₆ conjugates. European Society for Photobiology, Stresa, Italy; 1997.
16. Hasan T, Duska LR, Miller J, and **Hamblin MR**, Photodynamic therapy with a photoimmunoconjugate in combination with cisplatin administration for the treatment of advanced epithelial ovarian cancer. American Society of Clinical Oncology 34th Annual Meeting, Los Angeles, CA, 1998.
17. Rizvi I, **Hamblin MR**, Miller JL, Ortel B, Maytin EV, and Hasan T, Selective targeting of intraperitoneal tumors, 7th Biennial Congress of the International Photodynamic Association, Nantes, France, 1998.
18. **Hamblin MR**, Miller JL, Rizvi I, Ortel B, Maytin EV, and Hasan T, Mechanism of differential phototoxicity after pegylation of poly-l-lysine chlorin e6 conjugates. American Society for Photobiology, Snowbird, UT, 1998: Photochem Photobiol 1998; 67: 24S.
19. **Hamblin MR**, Penta P, Ortel B, and Hasan T, Targeting phototoxicity to macrophages via albumin photosensitizer conjugates. American Society for Photobiology, Snowbird, UT; 1998: Photochem Photobiol 1998; 67: 92S.
20. Goslee M, Khadem J, Tolentino F, **Hamblin MR**, and Hasan T, Photodynamic tissue adhesion with chlorin (e6) protein conjugates, Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, FA 1999: Invest Ophthalmol Vis Sci 1999; 40: S341

21. **Hamblin MR**, O'Donnell D, Murthy N, Rizvi I, and Hasan T. Targeted photoinactivation of gram negative bacterial contamination in wounds, European Society for Photobiology, Granada, Spain; 1999
22. Hasan T, Moor ACE, Ortel B, Rizvi I, and **Hamblin MR**, Targeted photosensitization, subcellular targeting and cell death, European Society for Photobiology, Granada, Spain; 1999
23. Hasan T, **Hamblin MR**, Moor ACE, Ortel B, Rizvi I, Savellano M. Selective subcellular localization of photosensitizers and consequent phototoxicity, 3rd International Symposium on Photodynamic Diagnosis and Therapy in Clinical Practice, Innsbruck, Austria, October 1999.
24. Kodama T, **Hamblin MR**, and Doukas, AG, Cytoplasmic molecular delivery with shock waves, American Society for Biochemistry and Molecular Biology and American Society for Pharmacology and Experimental Therapeutics Joint Meeting, Boston MA, 2000. FASEB J. 14, A1473, 2000
25. Soukos NS, **Hamblin MR**, Deutsch TF, Fabian RL, Keel S, Hasan T. Epidermal growth factor receptor targeted immunophotodiagnosis of oral precancer. IADR/AADR/CADR 78th General Session, Washington DC, 2000. J Dent Res, 79, SI165, 2000
26. **Hamblin MR**, O'Donnell DA, Murthy N, Rajagopalan K, Sherwood ME and Hasan T, Photoinactivation of antibiotic-resistant bacteria using polycationic photosensitizer conjugates, 13th International Congress on Photobiology, San Francisco, CA, 2000
27. **Hamblin MR**, O'Donnell DA, Murthy N, Contag CH, McManus AT, and Hasan T, Photodynamic inactivation of luminescent bacteria in contaminated and infected wounds in the mouse, 13th International Congress on Photobiology, San Francisco, CA, 2000
28. **Hamblin MR**, Del Governatore M, Shea CR, Rizvi I, Molpus KL, and Hasan T, Experimental photoimmunotherapy for treatment of hepatic metastases of colorectal cancer in the mouse, 13th International Congress on Photobiology, San Francisco, CA, 2000

29. **Hamblin MR**, Huzaira M, and O'Donnell DA, Scavenger receptor-targeted photodynamic therapy of murine tumors in vivo, 13th International Congress on Photobiology, San Francisco, CA, 2000
30. Hasan T, **Hamblin MR**, Moor AC, Ortel B, Rizvi I, and Savellano M, Targeted photosensitization in the optimization of PDT 13th International Congress on Photobiology, San Francisco, CA, 2000
31. Hasan T, **Hamblin MR**, Moor ACE, Ortel B, Rizvi I, Savellano M. Strategies for enhanced selectivity in photosensitization., First International Conference on Porphyrins and Phthalocyanines (ICPP-1) PDT Symposium, Djion, France, 2000.
32. **Hamblin MR**, O'Donnell DA, Murthy N, Contag CH, McManus AT, and Hasan T, Photodynamic therapy for wound infections. 8th International Photodynamic Association Congress, Vancouver, BC, Canada, 2001
33. **Hamblin MR**, Huzaira M, and O'Donnell DA, Scavenger receptor-targeted photodynamic therapy of murine tumors in vivo, 8th International Photodynamic Association Congress, Vancouver, BC, Canada, 2001
34. Okumura A, **Hamblin MR**, Hasan T, Ishikawa T, Kakumu S, Takahashi H, Apoptosis is induced by caspase activation and cytochrome c release in photodynamic therapy (PDT) of human hepatocellular carcinoma cells. *Gastroenterology*, **120**: 1866 Suppl, 2001.
35. **Hamblin MR**, O'Donnell DA, Zahra T, Contag CH, McManus AT, and Hasan T. Targeted photodynamic therapy for infected wounds in mice. *Optical Techniques for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XI*, BioS 2002 Biomedical Optics, Photonics West, San Jose, CA, 2002.
36. **Hamblin MR**, O'Donnell DA, Huzaira M, and Zahra T. Scavenger receptor-targeted photodynamic therapy of J774 tumors in mice: tumor response and concomitant immunity (Invited paper). *Laser Tissue Interaction XIII: Photochemical, Photothermal, Photomechanical*, BioS 2002 Biomedical Optics, Photonics West, San Jose, CA, 2002.

37. Tawakol A, Fischman AJ, Hasan T, Zahra T, Muller JE, and **Hamblin MR**
Accumulation of a novel macrophage-targeting photodynamic compound within lipid-rich atherosclerotic plaques, 51st Annual Meeting of the American College of Cardiology, ACC-02, #842-2, Atlanta, GA, 2002.
38. **Hamblin MR**, Tawakol A, Ahmadi A, Zahra T, Syed S, Ortel B, Fischman AJ, and Muller JE. Scavenger receptor-targeted photodynamic therapy for diagnosis and treatment of vulnerable atherosclerotic plaques. 30th Annual Meeting of the American Society for Photobiology, Quebec City, Canada, 2002.
39. **Hamblin MR**, Ahmadi A, Tolokoff MJ, and Zahra T, Light-mediated killing of *Helicobacter pylori* in vitro and ex vivo. 30th Annual Meeting of the American Society for Photobiology, Quebec City, Canada, 2002.
40. **Hamblin MR**, Zahra T, Francis KP, and Hasan T. Targeted photodynamic therapy for *Staphylococcus aureus* soft tissue infections in vivo monitored by bioluminescence imaging. 30th Annual Meeting of the American Society for Photobiology, Quebec City, Canada, 2002.
41. **Hamblin MR**, Zahra T, Hasan T, and Francis KP. Use of genetically engineered bioluminescent bacteria to develop animal models of localized infections suitable for photodynamic therapy. International Quantum Electronics/Laser Application Technology 2002 Meeting, Moscow, Russia, 2002
42. **Hamblin MR**, Miller JL, Rizvi I, Ortel B, and Hasan T. Degree of substitution of chlorin(e6) conjugated to charged poly-L-lysine chains affects their cellular uptake, localization and phototoxicity. Saratov International Workshop on Biophotonics – SIWB02 Saratov, Russia, 2002
43. **Hamblin MR**, Tawakol A, Ahmadi A, Zahra T, Syed S, Ortel B, Fischman AJ, and Muller JE. Scavenger receptor-targeted photodynamic therapy for diagnosis of vulnerable atherosclerotic plaques. Saratov International Workshop on Biophotonics – SIWB02 Saratov, Russia, 2002

44. **Hamblin MR**, Whitaker SJ, O'Donnell DA, and Zahra T, Photodynamic therapy of mouse tumors; local control and anti-tumor immunity. (Invited paper). Laser Tissue Interaction XIV: Photochemical, Photothermal, Photomechanical, BioS 2003 Biomedical Optics, Photonics West, San Jose, CA, 2003.
45. **Hamblin MR**, Tawakol A, Ahmadi A, Zahra T, Shirazi A, Stern J, Syed S, Ortel B, Fischman AJ, and Muller JE. Macrophage-targeted photodynamic detection of vulnerable atherosclerotic plaque. Diagnostic and Therapeutic Cardiovascular Interventions XII, BioS 2003, Biomedical Optics, Photonics West, San Jose, CA, 2003.
46. Hamrahi V, **Hamblin MR**, Carter EA, Benjamin J, Francis KP, Fischman AJ, Tompkins RG. A novel method for monitoring bacterial growth in burn eschar. 35th Annual Meeting American Burn Association, Miami Beach, FL, 2003.
47. **Hamblin MR**, Castano AP, Gad F, and Zahra T. Photodynamic therapy mediated by benzoporphyrin derivative and chlorin(e6) generates tumor-specific immune response against EMT-6 tumors in Balb/c mice. 94th Annual Meeting of the American Association for Cancer Research, Toronto, Canada, 2003.
48. Tawakol A, Fischman A, Gewirtz H, Muller JE, Brady T, **Hamblin MR**, Intravascular detection of inflamed atherosclerotic plaques with a novel macrophage-targeted fluorescent photodynamic compound. ACC-03, Chicago, IL, 2003.
49. Ganz R, **Hamblin MR**, Tolckoff MJ, Ahmadi A, Zahra T. *Helicobacter pylori* and *Helicobacter mustelae* are killed by visible light, in-vitro and ex-vivo. Digestive Disease Week 2003, Orlando, FL, 2003.
50. **Hamblin MR**, Castano AP, Liu Q. Induction of anti-tumor immunity by photodynamic therapy of mouse tumors. 31st Annual Meeting of American Society for Photobiology, Baltimore, MD, 2003
51. **Hamblin MR**, Tawakol A, Migrino RQ, Chirico S, Castano AP, Gad F, Ahmadi A, Zahra T, Shirazi A, Stern J, and Muller JE. Macrophage-targeted photoactive molecules localize in vulnerable atherosclerotic plaques in vivo. 31st Annual Meeting of American Society for Photobiology, Baltimore, MD, 2003.

52. **Hamblin MR**, Demidova T, Photodynamic destruction of anthrax-like spores, 10th Congress of the European Society for Photobiology, Vienna, Austria, 2003.
53. **Hamblin MR**, Ganz RA, Viveiros J, Ahmadi A, Tolckoff MJ. Phototherapy for *Helicobacter pylori* infection: in vitro, ex vivo and Phase I clinical trial. 10th Congress of the European Society for Photobiology, Vienna, Austria, 2003.
54. Gad F, Zahra T, Hasan T, and **Hamblin MR**. Targeted photodynamic therapy of established soft-tissue infections in mice. Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy, BioS 2004, Biomedical Optics, Photonics West, San Jose, CA, 2004.
55. Castano AP, Liu Q, and **Hamblin MR**. Photodynamic therapy cures green fluorescent protein expressing RIF1 tumors in mice. Laser Interaction with Tissue and Cells XV, BioS 2004, Biomedical Optics, Photonics West, San Jose, CA, 2004.
56. Castano AP and **Hamblin MR**. Combination of photodynamic and low-dose cyclophosphamide as a treatment for metastatic murine tumors. 95th Annual Meeting of the American Association for Cancer Research, Orlando, FL 2004.
57. **Hamblin MR** and Castano AP. Anti-tumor immunity generated by photodynamic therapy in a metastatic murine tumor. 32nd Annual Meeting of American Society for Photobiology, Seattle, WA, 2004
58. Demidova TN and **Hamblin MR**. Strong microbe-photosensitizer binding leads to efficient photoinactivation. 32nd Annual Meeting of American Society for Photobiology, Seattle, WA, 2004.
59. **Hamblin MR**, Ganz RA Viveiros J, Zalesky P, Kelly C, and Tolckoff MJ. Phototherapy for *Helicobacter pylori* infection: in vitro, and Phase I clinical trial. European Helicobacter Study Group XVII International Workshop, Vienna, Austria. 2004

60. Hamblin MR, Demidova TN, Gad F, Zahra T, Hasan T. Photodynamic modulation of wound healing and inhibition of tissue degradation. Light Activated Tissue Regeneration and Therapy I, Engineering Conferences International, Kona, HI, 2004
61. **Hamblin MR**, Anatelli F, Khadem JJ, and Hasan T. Photodynamic tissue repair and healing. Light Activated Tissue Regeneration and Therapy I, Engineering Conferences International, Kona, HI, 2004
62. Tegos GP, Demidova TN and **Hamblin MR**. Phenothiazinium photosensitizers are designated substrates of microbial multidrug resistance pumps (MDRs). 44th ICAAC Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.
63. Demidova TN, Tegos GP, and **Hamblin MR**. Anthrax surrogate spores can be destroyed with photodynamic therapy. 44th ICAAC Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.
64. Koshiyama K, Yano T, **Hamblin MR**, Doukas AG, Fujikawa S, and Kodama T. Molecular Delivery into Lipid Bilayer with Impulsive Pressure using Molecular Dynamics Simulation. 4th International Symposium on Therapeutic Ultrasound, Kyoto, Japan, 2004.
65. **Hamblin MR**, Gad F, Anderson RR, Hasan T. Photons for therapy: Targeted photodynamic therapy for infected and contaminated wounds. NATO Research and Technology Organization Symposium: Combat Casualty Care in Ground Based Tactical Situations: Trauma Technology and Emergency Medical Procedures. HFM-109 /RSY organized by the Human Factors and Medicine Panel in cooperation with the US Department of Defense Advanced Technology Applications for Combat Casualty Care Conference. *St Pete Beach FL, 2004.*

Patents issued:

Trauner KB, Hasan T, **Hamblin MR**, inventors; Massachusetts General Hospital assignee. Inhibition of fibrosis by photodynamic therapy. US Patent 5,913,884. 1999 Jun 22.

Hasan T, **Hamblin MR**, Trauner KB, inventors; Massachusetts General Hospital assignee. Acceleration of wound healing by photodynamic therapy. US Patent 6,107,466. 2000 Aug 22.

Hasan T, **Hamblin MR**, Soukos, NS, inventors; Massachusetts General Hospital assignee. Photosensitizer conjugates for pathogen targeting. US Patent 6,462,070. 2002 Oct 8.

Hamblin MR, Khadem JJ, inventors. Massachusetts General Hospital assignee. Methods for tissue welding using laser-activated protein solders. US Patent 6,607,522. 2003 Aug 19.

Patents pending:

Hamblin MR, Tawakol A, Hasan T, Muller J, Fischman AJ, Anderson RR. inventors. Detection and therapy of Thin Capped Fibro-Atheroma with Photodynamic Compounds that Target Components of the Vulnerable Plaque. United States Patent Application 20030103995, June 5, 2003.

Fischman A, **Hamblin MR**, Tawakol A, Hasan T, Muller J, Anderson R, Elmaleh D, inventors. Methods and devices for detection and therapy of atheromatous plaque, United States Patent Application 20030082105, May 1, 2003

Invention disclosures:

Hamblin MR, inventor. Photodynamic therapy targeted to macrophages as a therapy for cancer and other diseases. Disclosed Mar 2001.

Hamblin MR, Khadem JJ, inventors. Cavitation bubbles produced by focused laser pulses to treat retinal vascular occlusion. Disclosed Mar 2001.

Hamblin MR, Kodama T, inventors. Shock waves generated by explosive micro-pellet for introduction of macromolecules into cells, tissues and lumens of vessels in vivo. Disclosed Mar 2001.

Hamblin MR, Hasan T, inventors. Targeted photodynamic therapy to destroy extracellular virulence factors of pathogenic microorganisms. Disclosed May 2001.

Tawakol A, Migrino, RQ, **Hamblin MR**, inventors. Enhanced detection and therapy of inflamed tissues using immune modulation. Disclosed Oct 2002.

Hamblin MR and Demidova T, inventors, Photodynamic destruction of bacterial spores, MGH2399, disclosed Apr 2003.

Hamblin MR, Cope R, and Hasan T, inventors, Photodynamic therapy for Buruli ulcer disease, MGH2459, disclosed Sep 2003.

Hamblin MR, Gad F, and Hasan T, inventors, Second generation polycationic photosensitizer conjugates for PDT of infectious disease, disclosed Oct 2003.

Hamblin MR and Tegos GP. Potentiation of antimicrobial photodynamic therapy by inhibitors of microbial multidrug resistance pumps, Disclosed Aug 2004.